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(57) Abstract

The present invention relates to conjugates of carboxy gem-bisphosphonic acids with alkylating agents. Such derivatives are endowed with remarkable antitumor activity and with specific activity on bone resorption. The present invention also relates to a process for preparing them and to pharmaceutical compositions containing them.

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DERIVATIVES OF CARBOXY GEM-BISPHOSPHONATES WITH ANTITUMOR ACTIVITY, A PROCESS FOR PREPARING THEM AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

The present invention relates to conjugates of carboxy gem-bisphosphonic acids with alkylating agents. Such derivatives are endowed with remarkable antitumor activity and with specific activity on bone resorption. The present invention also relates to a process for preparing them and to pharmaceutical compositions containing them.

The skeletal system is the third more common site of metastases and more than 80% of all dead cancer patients show bone tumors at autopsy. Bone metastases account for a significant proportion of cancer-related morbidity, causing derangement in calcium metabolism and bone marrow involvement and are responsible for the sevre consequences in patients with cancer, such as pain, pathological fractures, compression of the spinal cord and hypercalcemia (Drew et al., Osseous complication of malignancy, Lokich, J.J. ed. Clinical cancer medicine: treatment tactics Boston: G.K. Hall Medical Publisher, 1980, 97-112).

The bone lesions are one of the most important features associated with bone tumors. Most of the local lesions caused by bone metastases are known to be a direct effect on the mineralized matrix or an indirect effect of tumor-stimulated bone resorption. The process of bone resorption is mediated predominantly by multinucleated osteoclasts and involves the release of bone mineral and the degradation of bone matrix. The

osteoclasts resorb the bone across a specialized area of the cell membrane known as "ruffled border". The resorption of bone is associated with the release of lysosomal enzymes and collagenases by the osteoclasts, as well as with the local production of acid which is responsible for causing release of mineral from the bone (Mundy, G.R., Bone resorption and turnover in health and disease, Bone, 1987, 8, S9-S16).

Therefore it appears that the discovery of a drug able to inhibit both tumor growth and bone destruction is a primary target in antitumor research.

Gem-diphosphonic acids and salts thereof are known and employed in the therapy of osteoporosis and in the treatment of bone resorption (see EP 96.931, EP 252.504, BE 896.453, BE 903.519, DE 3.016.289, DE 3.540.150, DE 2.534.391, DE 3.512.536). However, for none of the above compounds antitumor activity is described.

DE 3.425.812 (Blum et al.) describes derivatives of 1,1-diphosphonic acids, characterized by a bis[(halogenoalkyl)amino]phenyl residue, as agents useful in the treatment of bone tumors. The bone tropism typical of the diphosphonic acids is in fact coupled with the cytotoxic activity typical of molecules carrying dialkylating functionality. No activity on bone resorption is however described.

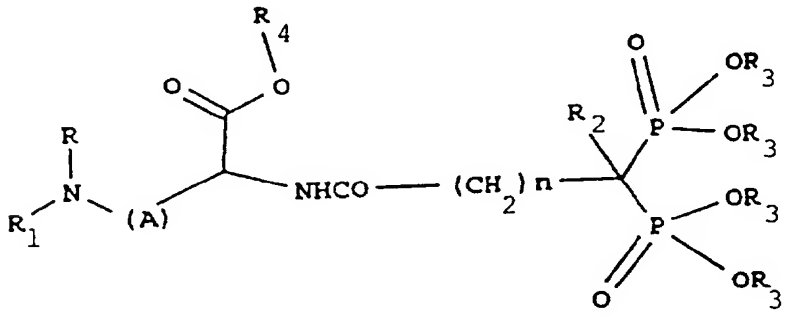
Furthermore, in WO 88/06158 diphosphonic analogues of metotrexate are described as agents useful in the treatment of bone tumors.

Therefore it appears that none of the above mentioned compounds has shown efficacy in acting contemporaneously both as antitumor agent and as

inhibitor of bone resorption. Particularly, the compounds disclosed in DE 3.425.812, even if they are conjugates between a diphoshonate, active on bone resorption, and an alkylating, antitumor agent, have maintained only the latter activity. This demonstrates that the activity of the final compound is not automatically foreseeable simply adding the corresponding activities of the two starting intermediates.

WO 92/18512 claims however conjugates between bis-phosphonic acids and alkylating agents characterized in having the two molecules linked by means of amino acid moieties. The amide bond, which can probably be metabolically hydrolyzed, is said to be responsible for the activity of these compounds, which maintain the efficacy both as antitumor agents and as inhibitors of bone resorption. However said molecules are not able to completely inhibit the bone resorption, especially after long times from treatment (see Table I).

We have now found that the compounds of the general formula (I):

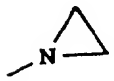


(I)

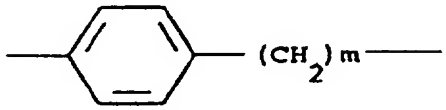
wherein:

R and R_1 represent halo-ethyl (2-chloroethyl, 2-bromoethyl, 2-iodoethyl) or, taken together with the

nitrogen atom to which they are linked, are a 1-aziridinyl residue of formula



(A) is linear or branched (C_1-C_5)alkylene, phenylene or an aralkyl chain of formula



wherein m is an integer between 1 and 5;

n is an integer between 1 and 6;

R_2 is hydrogen or a hydroxy group;

R_3 hydrogen or (C_1-C_4)alkyl;

R_4 represents hydrogen or (C_1-C_4)alkyl,

are endowed with a remarkable antitumor activity and contemporaneously are able to totally inhibit the bone resorption even after long time from treatment.

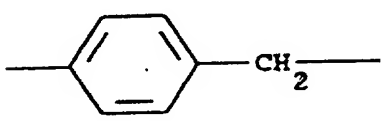
The diastereoisomers, the racemates and the pure enantiomers of the compounds of formula (I) are encompassed in the scope of the present invention.

In the scope of the present invention are furthermore encompassed the pharmaceutically acceptable salts of the compounds of formula (I), such as those with inorganic bases such as salts with alkali (for example sodium or potassium) or alkaline-earth metal ions (for example calcium or magnesium) or ammonium salts; the salts with organic bases such as methylamine, ethylamine, propylamine, isopropylamine, butylamine, tert-butylamine, dimethylamine, diethylamine, dietha-

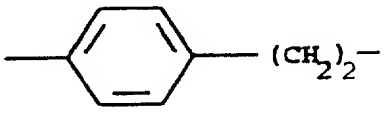
nolamine, trimethylamine, triethylamine, piperidine, pyridine, picoline, dicyclohexylamine; the salts with inorganic or organic acids such as hydrochloric, hydrobromic, sulfuric, phosphoric, nitric, formic, acetic, trifluoroacetic, maleic, fumaric, tartaric, methanesulphonic or paratoluenesulphonic acid; the salts with amino acids such as aspartates, glutamates or salts with lysine or arginine.

R and R_1 are preferably a 2-haloethyl group;

(A) is preferably a group of formula



or

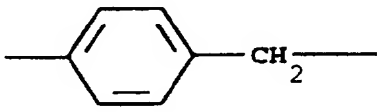


n is preferably the integer 2 or 3;

R_2 is preferably a hydroxy group;

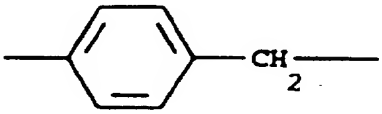
R_3 and R_4 are preferably hydrogen.

Particularly preferred compounds are those in which R and R_1 are a haloethyl group, (A) is a group of formula



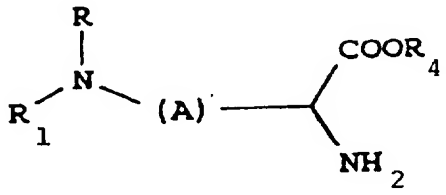
n is the integer 2 or 3 and R_3 and R_4 are hydrogen.

Most preferred compounds are those in which R and R_1 are a 2-chloroethyl group, (A) is a group of formula



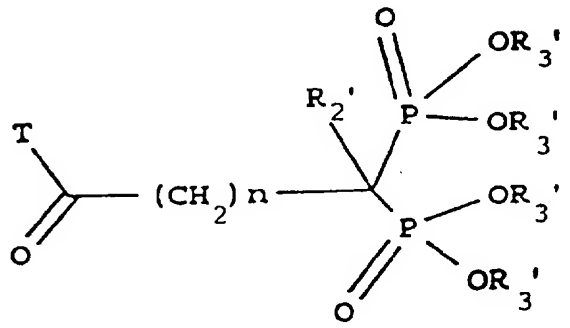
n is the integer 2 or 3, R_2 is a hydroxy group and R_3 and R_4 are hydrogen.

The compounds of the general formula (I) can be prepared following a process which comprises the condensation reaction of a compound of formula (II):



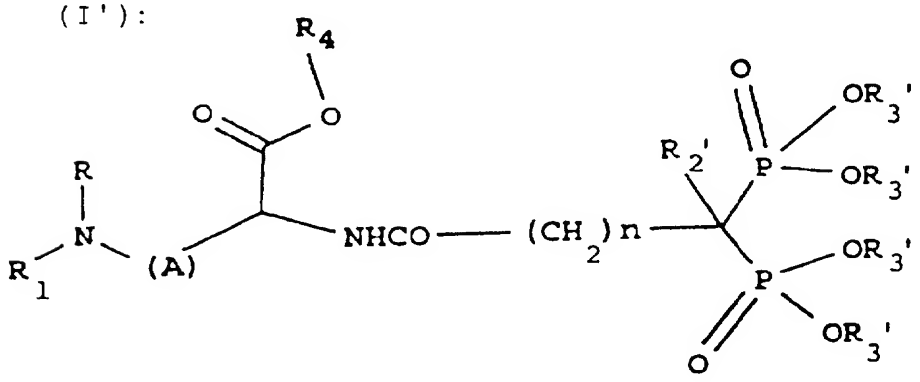
(II)

wherein R, R_1, (A) and R_4 have the above meanings, with a bis-phosphonate of formula (III):



(III)

wherein n has the above meaning, R_2' has the above meanings or is a O-G group, in which G is a suitable protecting group for a tertiary alcohol; R_3' has the above meanings except hydrogen; T is a hydroxy or a carboxy-activating group, to give compounds of formula (I'):



(I')

which can be converted into the compounds of formula (I) by removal of the protecting groups optionally present and/or by optional hydrolysis of the phosphonic esters to give the corresponding phosphonic acids and optional salification of the obtained compounds with pharmaceutically acceptable acids or bases.

Diastereoisomers of the compounds of formula (I) optionally present may be separated by selective crystallization or by purification via liquid chromatography.

Enantiomers of the compounds of formula (I) optionally present may be separated from the racemic mixtures following methods of optical resolution known to those skilled in the art, while not obtained directly in the synthesis starting from optically active reagents.

The protecting groups to which reference is made in the present invention are all protecting groups for an alcoholic or carboxylic oxygen atom, such as ethers, esters and silyl derivatives.

Preferred examples of G protecting groups are silyl ethers and particularly tertbutyl dimethyl silyl ether.

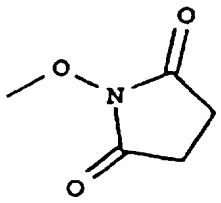
Such protecting groups can be removed by means of reaction well known to the skilled artisan, such as removal in basic conditions in the case of esters and removal in acidic conditions in the case of ethers and silyl derivatives. The phosphonic esters can be hydrolyzed with particular selective agents, such as trimethylsilyl iodide.

When in the reaction of compounds of formula (II) with compounds of formula (III) these latter are used as

free carboxylic acids (T = OH), the reaction is generally conducted in the presence of a condensing agent such as N,N'-dicyclohexyl carbodiimide, N-cyclohexyl-N'-morpholinoethyl carbodiimide, N-ethyl-N'-(3-dimethylamino)propyl carbodiimide, N,N'-carbonyl bis-(imidazole), phosphorus oxychloride, phosphorus trichloride, thionyl chloride, oxalyl chloride, ethyl-chloroformate, isobutylchloroformate, morpholinoethylisonitrile or similar reagents.

When T is a carboxy-activating group , examples of -C(=O)T groups are acyl halides, symmetrical or mixed anhydrides (for example with methanesulfonic, acetic, isobutyric, pivalic, trifluoroacetic acids); activated amides (for example with imidazole, 1,2,4-triazole); azide; activated esters (for example paranitrophenyl ester, methoxymethyl ester, 2,4-dinitrophenyl ester, pentachlorophenyl ester, hydroxysuccinimido ester, 1-hydroxy-2-(1H)-pyridone ester, 1-hydroxybenzotriazole ester) and similar groups.

A particularly preferred activating T group is the hydroxysuccinimidyl group of formula



The condensation reaction of the compounds of formula (II) with the compounds of formula (III) can be performed in the presence of an inorganic base such as an alkali carbonate or bicarbonate, an alkali or alkaline-earth hydroxide or of an organic base such as

triethyl amine, tributyl amine, pyridine, 4-dimethylamino pyridine, N-alkylmorpholine, N,N-dialkylaniline or similar bases. The pH is preferably maintained not above pH = 9.

The reaction temperature can range from -40°C to the boiling temperature of the solvent, according to the chosen activating group, preferably between -10°C and 50°C.

The preferred solvents are inert organic solvents, such as pyridine, N,N-dimethyl formamide or acetonitrile, or mixtures thereof with water in various proportions.

The reaction times are variable with the activating group and the substrate which are chosen and can be comprised between 30 minutes and 48 hours.

Particularly preferred reaction conditions are those which provide for the use of triethyl amine, in molar excess on the reagents, in a 1:10 water/acetonitrile mixture and at a temperature comprised between 0°C and room temperature.

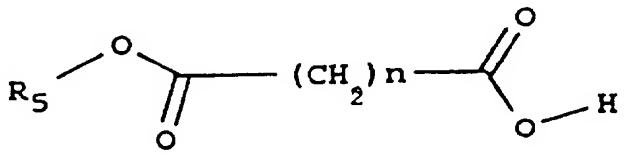
The compounds of formula (II) are known compounds, commercially available and/or preparable with methods known to those skilled in the art, such as those described in : J. Med. Chem., 24, 1304 (1981); CA 51: 8066d (1957); BE 905.974; CA 104: 141897 (1986); J. Med. Chem., 7, 468 (1964); J. Med. Chem., 6, 85 (1963); Cancer Chem. Rep., 50, 685 (1966); J. Med. Chem., 21, 16 (1977); J. Org. Chem., 26, 1554 (1961); J. Org. Chem., 26, 1674 (1961); CA 64: 10267g (1966); J. Chem. Soc., 2994 (1960); Biochem. Pharmacol., 11, 847 (1962); Biochem. Pharmacol., 12, 833 (1963); CA 73: 131293c

(1970); Biochem. Pharmacol., 5, 192 (1960); Int. J. Pept. Protein Res., 36, 308 (1990).

The compounds of formula (III) in which R_2' is hydrogen and T is a OH group are known compounds or can be prepared according to experimental methods known to those skilled in the art [Synthesis, 661 (1991); Phosphorus, sulfur, Silicon Relat. Elem., 88(1-4), 1-13 (1994); FR 2683527; Zh. Obshch. Khim., 61(12), 2698 (1991); Bioorg. Khim., 12(9), 1282 (1986); J. Organometal. Chem., 13(1), 199 (1968)].

The compounds of formula (III) in which R_2' is O-G (wherein G is hydrogen or a suitable protecting group) and T is a OH group can be synthesized according to the following process comprising the steps of:

(a) reacting equimolar amounts of a cyclic anhydride of a suitable dicarboxylic acid and of an alcohol of formula R_5-OH, wherein R_5 is an alkyl group of 1 to 4 carbon atoms or is a benzyl group, optionally substituted, or allyl group, obtaining the intermediate of formula (IV):



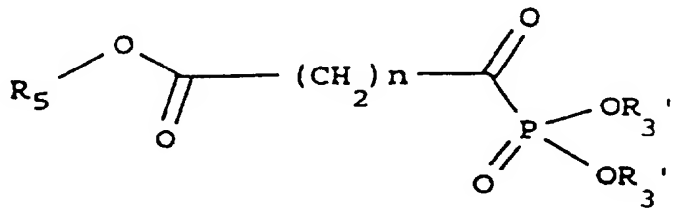
(IV)

The reaction is performed in an inert solvent, preferably dimethyl formamide, in the presence of a base, preferably pyridine, and at a temperature ranging from room temperature to the boiling point of the reaction mixture;

(b) suitably activating the non-esterified carboxylic group present in intermediate (IV), transforming it

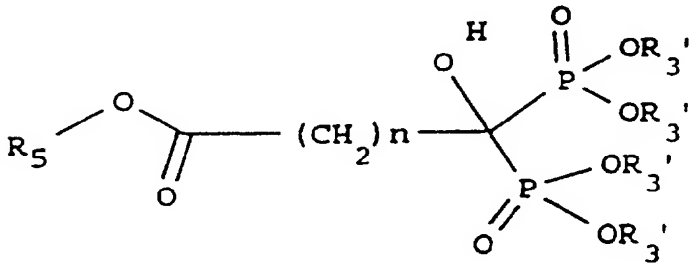
for example into an acyl halide, an anhydride or an activated ester such as hydroxysuccinimido ester. A preferred example of the activation of the carboxylic group in intermediate (IV) is its transformation into acyl chloride by means of thionyl chloride;

(c) reacting the intermediate obtained in step (b) with a trialkyl phosphite $P(OR_3')_3$ (preferably trimethyl phosphite) in an inert solvent such as chloroform and at a temperature ranging from -10°C to room temperature, obtaining the intermediate of formula (V):



(V)

(d) reacting the intermediate of formula (V) with a dialkyl phosphite $HP(O)(OR_3')_2$ (preferably dimethyl phosphite) in the presence of a base, preferably an organic base such as dialkyl or trialkyl amines, obtaining the intermediate of formula (VI):



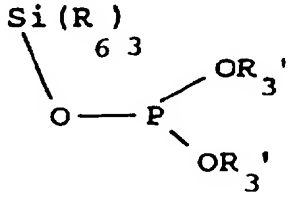
(VI)

(e) optionally protecting the OH group with a suitable protecting group selected from those suitable for a

tertiary alcohol;

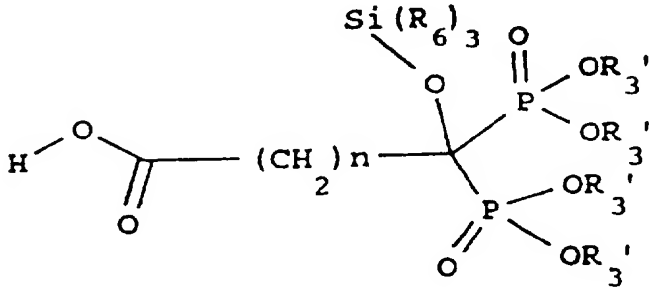
(f) deprotecting the $-COOR_5$ ester group by means of hydrolysis reaction in basic or acid medium and in conditions in which the other R_3' protecting groups and the group optionally present on the alcoholic functionality are not affected or, in the case R_5 is a benzyl or allyl group, by catalytic hydrogenation or in the presence of hydrogen donors such as ammonium formate or sodium hypophosphite.

A particularly advantageous alternative process for obtaining the intermediates of formula (III) wherein R_2' is a -O-silyl group is that which replaces in step (d) the dialkyl phosphite with a dialkyl silyl phosphite of formula (VII):



(VII)

obtaining directly the intermediate of formula (III'):



(III')

wherein the R_6 groups can be the same or different. A particularly preferred silyl group is the tertbutyl dimethyl silyl group.

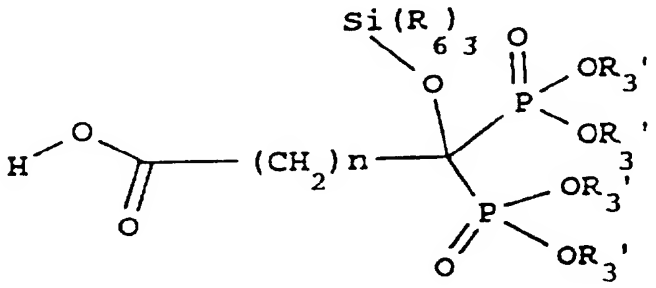
Such a process allows to obtain in one step the intermediate already protected at the alcoholic oxygen,

thus avoiding the step (e) of oxygen protection.

The intermediates of formula (III) in which both T and R_2' are OH groups are known (DE 2117880; NL 6610762).

The intermediates of formula (III) in which T is a OH group and R_2' is a $-O-Si(R_6)_3$ group are new.

A further object of the present invention are therefore the intermediates of formula (III) with T = OH and R_2' = $-O-Si(R_6)_3$, that is the intermediates of formula (VIII):



(VIII)

wherein n and R_3' have the above meanings and the R_6 groups, which can be the same or different, are selected from the group comprising linear or branched (C_1-C_4)alkyl or phenyl.

A particularly preferred meaning of $Si(R_6)_3$ group is tertbutyl dimethyl silyl group.

The derivatives of formula (III) in which T is a suitable activating group are obtained from the derivatives with T = OH by means of reactions well known to those skilled in the art.

The compounds of the invention have been tested "in vivo" against Walker 256/B mammary carcinoma in the rat intratibially implanted. Once inoculated in the cavity of the tibia's bone marrow, said tumor grows inside the

bone causing osteolytic lesions, paraneoplastic hypercalcemia and it invades the surrounding tissues producing a measurable tumor mass. It is thus possible to measure both the antitumor activity against the extraosseous tumor and the antiosteolytic effect (Cancer, 72(1), 91 (1993)).

The tumor Walker 256/B has been obtained by NCI Frederick Cancer Facility and maintained in male rats CD1 by means of subcutaneous transplant of tumor fragments of about 1 cm diameter every 10 days.

To perform the experiment the rats are injected with $2.5x10^6$ tumor cells; the animals are anaesthesized with a mixture of 10 mg/kg of Ketalar (Park-Davis) and 5 mg/kg of Rompun (Bayer). Once transplanted inside the tibia, the tumors grows causing osteolytic lesions associated with an increased activity of the osteoclasts. To obtain an injectable cell suspension, the tumor fragments are desegregated by means of enzymatic digestion using collagenase type IV (Sigma) at a concentration of 400 U/ml for 20 minutes at 37°C.

The compounds of the invention have been administered i.v. in the days 1, 4 and 7 after the tumor transplant. The extraosseous tumor mass has been measured at day 14 after the tumor transplant.

The antitumor activity has been determined as TWI% (tumor weight inhibition %) calculated according to the following formula:

TWI% =

$(100 - TW\ mean_{treated\ anim.}/TW\ mean_{controls}) \times 100$

wherein the TW for each animal is given by the formula:

$$TW = \frac{1}{2}(ab)^2$$

wherein a and b are respectively the maximum and minimum diameter of the tumor mass in millimeters.

The osteolytic lesions have been radiologically evaluated at days 8 and 16 following the tumor transplant. The animals have been anaesthesized, placed in prone position on a Rx-film (X-OMAT MA, S.p.A.). The bone lesion degree has been determined according to the following scores:

- absence of lesions
+/- low dihomogeneity
+ high dihomogeneity
++ isolated lesions
+++ wide lesions

On the base of such scores the Destruction Index (DI) has been calculated:

DI = no. of lesions x degree of lesion/total no. of animals.

DI values lower or equal to 1 mean a good bone protection. A value of DI = 0 means a complete absence of lesions and thus a total protection or repair.

The compounds of the invention have been tested on this experimental model with respect to the prior art compounds and have shown an higher activity not only as far as the antitumor activity against the extraosseous tumor is concerned, but also as antiosteolytic agents. Table I shows the comparison data for a molecule representative of the compounds of formula (I).

Table I

Antitumor activity of 4-[bis(2-chloroethyl)amino]-N-(5,5-diphospho-5-hydroxy-1-pentanoyl)-(L)-phenylalanine trisodium salt (example 1), 4-[4-(bis(2-chloroethyl)-amino)phenyl]-1-hydroxybutane-1,1-diphosphonic acid trisodium salt (compound A, Blum et al.), N-[[4-bis(2-chloroethylamino))-(L)-phenylalanyl]-(L)-alanyl]-4-amino-1-hydroxybutane-1,1-diphosphonic acid trisodium salt (compound B, WO 92/18512) against the Walker 256/B mammary carcinoma in the rat (it/iv; days 1,4,7).

Compound	dose(mg/kg)	TWI%	DI	
			day 8	day 16
Example	20	83	0.75	0.25
	30	84	0.0	0.0
A	55	67	2.2	---
B	20	80	0.6	0.7

Yet a dose of 20 mg/kg, the compound of the invention shows a continuous tendency to a bone lesions repair, with DI values which pass from 0.75 after 8 days to 0.25 after 16 days. At the same dosage, compound B shows on the contrary the opposite tendency, being detected an increase of the destruction index at day 16. This demonstrates that compound B is not able of causing a true long lasting bone tissue repair.

Furthermore, the compounds of the invention resulted active in an experimental model of human multiple myeloma on the laboratory animal.

The human multiple myeloma is a tumor which has as a target the plasma cells and it is sensitive to

alkylating agents such as melphalan. Bone damages are often associated to the tumor, causing pain and fractures. It appears therefore that a drug able to carry the alkylating agent to the bone and on the other hand to preserve or repair the bone from the damages caused by the tumor it is an extremely interesting target.

The test is performed by inoculating intravenously (i.v.) the human multiple myeloma HS-Sultan cells in SCID (immunodeficient) mice at day 0, followed by the i.v. treatment with a compound of the invention at days 15, 18 and 21.

The activity of the compounds of the invention has been evaluated with respect to the following parameters:

- Mean Survival Time (MST) in comparison with not treated mice (which have a MST of 30 days after the tumor inoculation);
- evaluation of the histologic parameters (bone marrow invasion and entity of the osteolytic lesions) according to the methods previously described.

The compounds of the invention are endowed with a low acute toxicity and are well tolerated in the animals.

The high water solubility of the compounds of the invention allows to prepare parenteral and oral pharmaceutical compositions.

The compounds of formula (I), when administered to men and animals carrying tumors susceptible of alkylating agent therapy, in variable dosages from 1 mg to 1200 mg per square meter of body surface, are able to

cause the regression of said tumors and to allow the bone tissue repair, avoiding in this way the pathological manifestations associated with the bone lesions.

The effective dosage of the compounds of the invention can be determined by an expert clinician according to known conventional methods.

The correlation between dosages used in the animals of various species and in the man (given in mg/m^2 of body surface) is described in Freirich, E.J. et al., Cancer Chemother. Rep., 50, n.4, 219-244, May 1966.

The tumors susceptible of therapy with the compounds of the present invention are those susceptible of therapy with alkylating agents.

In particular, can be advantageously treated multiple myeloma, osteosarcoma, bone metastasis, breast, ovary and testis carcinomas.

The pharmaceutical compositions containing the compounds of formula (I) are comprised in the scope of the invention.

Such pharmaceutical compositions may contain any amount of the compounds of formula (I) having antitumor activity in the mammals against tumors susceptible of alkylating agent therapy. The pharmaceutical compositions may contain, in addition to at least one compound of formula (I), pharmaceutically compatible excipients, in order to allow the administration by any route, such as oral, parenteral, intravenous, intradermal, subcutaneous or topic routes, in liquid or solid form.

An administration route of the compounds of formula

(I) is the oral route. Oral compositions will generally include an inert diluent or an edible carrier. They may be included in gel capsules or compressed into tablets. Other forms suitable for the oral administration are capsules, pills, elixirs, suspensions or syrups.

The tablets, pills, capsules and similar compositions may contain the following ingredients (in addition to the active substance): a binder such as a microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose; a disintegrating agent such as alginic acid, primogel, corn starch and the like; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharine or a flavoring agent such as peppermint, methyl salicylate or orange flavor. When the chosen composition is in form of capsules, it may contain in addition a liquid carrier such as a fatty oil. Other compositions may contain other various materials which modify the physical form, such as coating agents (for tablets and pills) such as sugar or shellac. The materials used in the preparation of the compositions should be pharmaceutically pure and not toxic at the employed dosages.

For the preparation of pharmaceutical compositions by parenteral administration route, the active ingredient may be incorporated into solutions or suspensions, which may include in addition the following components: a sterile diluent such as water for injection, saline solution, oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol;

antioxidants such as ascorbic acid or sodium bisulphite; chelating agents such as ethylenediaminotetraacetic acid; buffers such as acetates, citrates or phosphates and agents for adjusting the solution tonicity such as sodium chloride or dextrose. The parenteral preparation may be included in ampoules, disposable syringes or glass or plastic vials.

The invention is further described by the following examples and preparations.

PREPARATION 1 - mono benzyl glutaric acid

A solution of glutaric anhydride (22.8 g) in benzyl alcohol (20.7 ml), anhydrous pyridine (250 ml) and dimethylformamide (25 ml) is refluxed for about 4 hours 30 minutes, then the solvent is evaporated off under reduced pressure and the residue is redissolved in 500 ml of ethyl acetate. The organic phase is then washed with 1 N hydrochloric acid (2x100 ml) and with water (3x100 ml), then it is basified with 200 ml of 1 N sodium hydroxide and with 200 ml of saturated solution of sodium hydrogen carbonate. The organic phase is separated, while the aqueous phase is neutralized by addition of 37% hydrochloric acid and extracted with methylene chloride (400 ml). The organic extracts are dried over sodium sulfate and the solvent is evaporated under reduced pressure, obtaining 32.8 g of product.

PREPARATION 2 - mono benzyl glutaroyl chloride

A solution of mono benzyl glutaric acid (32.8 g) in 250 ml of anhydrous toluene is added with 21.5 ml of thionyl chloride, then it is heated at 65°C for 1 hour. The solvent is evaporated off under reduced pressure and the residue is dissolved in toluene and evaporated,

repeating this step twice. 35 g of product as pale pink oil are obtained.

PREPARATION 3 - 5-(dimethoxyphosphinoyl)-5-ketopentanoic acid, benzyl ester

35 g of mono benzyl glutaroyl chloride are dissolved in 150 ml of anhydrous chloroform and the solution is cooled to 0°C. 18 ml of trimethyl phosphite are slowly dropped (during 1 hour 20 minutes), then the temperature is raised to room temperature. After an additional hour the solvent is evaporated off under reduced pressure obtaining 45 g of product as an oil.

PREPARATION 4 - (tertbutyldimethylsilyl)-dimethyl phosphite

To a suspension of 6.4 g of sodium hydride (60% in oil) in 255 ml of anhydrous tetrahydrofuran, cooled at 0°C, are added dropwise 13.4 ml of dimethyl phosphite, then the reaction mixture is refluxed for 2 hours 30 minutes. Afterwards, the temperature is raised to room temperature and 20 g of tert-butyldimethylsilyl chloride are added. The reaction mixture is again refluxed for a total of 16 hours, then the sodium chloride which separates is filtered off and the solvent is evaporated under reduced pressure.

23.62 g of product are obtained.

PREPARATION 5 - 5,5-(bis-dimethoxyphosphinoyl)-5-(tert-butyldimethylsilyloxy)pentanoic acid, benzyl ester

12.8 g of (tertbutyldimethylsilyl)-dimethyl phosphite are dropped into 18 g of 5-(dimethoxyphosphinoyl)-5-ketopentanoic acid, benzyl ester, cooled at 10°C. At the end of the addition, the temperature is raised to room temperature. After 4 hours

the reaction mixture is dissolved in 500 ml of ethyl acetate and the organic phase obtained is washed with 5% sodium hydrogen carbonate (3x70 ml), then with water (3x70 ml), then with 5% potassium carbonate (3x70 ml) and finally with a saturated solution of sodium chloride (70 ml). The organic phase is dried over sodium sulfate and the solvent is evaporated under reduced pressure, obtaining 25.9 g of residue which is purified by silica gel chromatography (eluent AcOEt - AcOEt/acetone 1:1) to give 17 g of the pure product as an oil.

PREPARATION 6

According to the methods described in preparations 1-5, starting from the suitable reagents and using the suitable trialkyl silyl halides, the following esters are prepared:

5,5-(bis-dimethoxyphosphinoyl)-5-(tertbutyldimethylsilyloxy)pentanoic acid, allyl ester;

4,4-(bis-dimethoxyphosphinoyl)-4-(tertbutyldimethylsilyloxy)butanoic acid, benzyl ester;

5,5-(bis-diethoxyphosphinoyl)-5-(tertbutyldimethylsilyloxy)pentanoic acid, benzyl ester;

5,5-(bis-dimethoxyphosphinoyl)-5-(trimethylsilyloxy)-pentanoic acid, benzyl ester;

3,3-(bis-dimethoxyphosphinoyl)-3-(tertbutyldimethylsilyloxy)propanoic acid, allyl ester;

8,8-(bis-dimethoxyphosphinoyl)-8-(tertbutyldimethylsilyloxy)octanoic acid, benzyl ester;

7,7-(bis-dimethoxyphosphinoyl)-7-(trimethylsilyloxy)heptanoic acid, benzyl ester;

5,5-(bis-diethoxyphosphinoyl)-5-(phenyldimethylsilyloxy)pentanoic acid, benzyl ester;

6,6-(bis-dimethoxyphosphinoyl)-6-(phenyldimethylsilyl-oxy)hexanoic acid, benzyl ester.

PREPARATION 7 - 4,4-bis(diethoxyphosphinoyl)butanoic acid, N-hydroxysuccinimido ester

To a solution of 4,4-bis(diethoxyphosphinoyl)-butanoic acid (1.28 g; prepared as described in Synthesis, 661-2 (1991)) and of N-hydroxy succinimide (0.613 g) in 35 ml of tetrahydrofuran are added dropwise 0.636 ml of morpholinoethyl isonitrile, while maintaining the temperature at about 0°C. The temperature is thus raised to room temperature and the reaction mixture is kept under stirring for 20 hours. After this time, the solvent is evaporated off under reduced pressure and the residue is redissolved in 70 ml ethyl acetate and washed first with water (40 ml), then with 1 N hydrochloric acid (40 ml) and with a saturated solution of sodium chloride (2x40 ml). The organic phase is dried over sodium sulfate and the solvent is evaporated off under reduced pressure to give 1.3 g of product.

EXAMPLE 1 - 5,5-bis(dimethoxyphosphinoyl)-5-(terbutyldi-methylsilyloxy)pentanoic acid

To a solution of 5,5-(bis-dimethoxyphosphinoyl)-5-(tertbutyldimethylsilyloxy)pentanoic acid, benzyl ester (4.5 g; preparation 5) in 110 ml of dimethylformamide are added portionwise 0.9 g of 10% palladium on carbon. The reaction mixture is hydrogenated under atmospheric pressure and at room temperature for 2 hours 45 minutes (consuming 0.372 ml of hydrogen). The catalyst is then eliminated by filtration through a celite plug and the solvent is evaporated off under reduced pressure. 4 g of

the product as a colorless oil are obtained.

N.M.R. (200 MHz) in $CDCl_3$: 3.85 ppm (m, 6H); 1.85-2.4 ppm (m, 6H); 0.9 ppm (s, 9H); 0.2 ppm (s, 6H).

EXAMPLE 2

According to the method described in example 1, starting from the ester of preparation 6, the following acids are obtained:

4,4-(bis-dimethoxyphosphinoyl)-4-(tertbutyldimethylsilyloxy)butanoic acid, benzyl ester;

5,5-(bis-diethoxyphosphinoyl)-5-(tertbutyldimethylsilyloxy)pentanoic acid, benzyl ester;

5,5-(bis-dimethoxyphosphinoyl)-5-(trimethylsilyloxy)-pentanoic acid, benzyl ester;

3,3-(bis-dimethoxyphosphinoyl)-3-(tertbutyldimethylsilyloxy)propanoic acid, allyl ester;

8,8-(bis-dimethoxyphosphinoyl)-8-(tertbutyldimethylsilyloxy)octanoic acid, benzyl ester;

7,7-(bis-dimethoxyphosphinoyl)-7-(trimethylsilyloxy)heptanoic acid, benzyl ester;

5,5-(bis-diethoxyphosphinoyl)-5-(phenyldimethylsilyloxy)pentanoic acid, benzyl ester;

6,6-(bis-dimethoxyphosphinoyl)-6-(phenyldimethylsilyloxy)hexanoic acid, benzyl ester.

EXAMPLE 3 - 4-[bis(2-chloroethyl)amino]-N-[4',4'-bis-(diethoxyphosphinoyl)-1'-butanoyl]-(L)-phenylalanine

To a suspension of 4-[bis(2-chloroethyl)amino]-(L)-phenylalanine (melphalan; 0.78 g) in 50 ml of dimethoxyethane and 0.575 ml of triethylamine is added dropwise a solution of 4,4-bis(diethoxyphosphinoyl)-butanoic acid, N-hydroxysuccinimido ester (1.3 g; preparation 7) in 10 ml of dimethoxyethane. After 24

hours under stirring at room temperature the solid completely dissolves. The solvent is then evaporated off under reduced pressure and the residue is redissolved in ethyl acetate (100 ml) and washed with 0.5 N (2x60 ml) and with a saturated sodium chloride solution (2x60 ml). The organic phase is dried over sodium sulfate and the solvent is evaporated off under reduced pressure, obtaining 1.6 g of residue which is purified by silica gel chromatography (eluents methylene chloride/methanol/acetic acid 95:5:1, then methylene chloride/methanol/acetic acid 90:10:1). 1.25 g of product are obtained.

N.M.R. (200 MHz) in $CDCl_3$: 7.12 ppm (d, 1H); 7 ppm (d, 2H); 6.55 ppm (d, 2H); 4.72 ppm (dd, 1H); 4.2 ppm (m, 8H); 3.6 ppm (m, 8H); 3 ppm (dd, 1H); 2.8 ppm (dd, 1H); 2.1-2.7 ppm (m, 4H); 1.35 ppm (m, 12H).

EXAMPLE 4 - 4-[bis(2-chloroethyl)amino]-N-[4',4'-diphospho-1'-butanoyl]-(L)-phenylalanine disodium salt

To a solution of the product of example 3 (1.1 g) in 35 ml of chloroform are added dropwise 2.24 g of trimethylsilyl iodide, maintaining the temperature at about 0°C. After 2 hours at 0°C and 1 hour at room temperature, the solvent is evaporated off under reduced pressure and the residue is redissolved in water (80 ml). The pH of the solution is adjusted to pH = 5 by addition of 1 N sodium hydroxide, then by addition of ethanol an oil separates which is removed by decantation. The oil obtained is crystallized from methanol, obtaining 0.9 g of product.

$[\alpha]_D$ = -2.6° (c = 1.05% in HCl 1 N)

N.M.R. (200 MHz) in D_2O: 7.2 ppm (d, 2H); 6.85 ppm (d,

2H); 4.37 ppm (dd, 1H); 3.75 ppm (s, 8H); 3.07 ppm (dd, 1H); 2.85 ppm (dd, 1H); 2.45-2.6 ppm (m, 2H); 1.75-2.25 ppm (m, 2H).

EXAMPLE 5

Following the methods described in preparation 7 and in examples 3-4, starting from the suitable reagents, the following gem-diphosphonic acids are obtained:

4-[bis(2-chloroethyl)amino]-N-[5',5'-diphospho-1'-pentanoyl](L)-phenylalanine disodium salt;

4-[bis(2-chloroethyl)amino]-N-[8',8'-diphospho-1'-octanoyl]-(L)-phenylalanine disodium salt;

4-[bis(2-bromoethyl)amino]-N-[4',4'-diphospho-1'-butanoyl](L)-phenylalanine disodium salt;

4-[bis(2-chloroethyl)amino]-N-[3',3'-diphospho-1'-propanoyl]-(L)-phenylalanine disodium salt;

4-[N'-aziridinyl]-N-[4',4'-diphospho-1'-butanoyl]-(L)-phenylalanine disodium salt.

EXAMPLE 6 - 5,5-bis(dimethoxyphosphinoyl)-5-(tertbutyldimethylsilyloxy)pentanoic acid, N-hydroxysuccinimido ester

To a solution of 5,5-bis(dimethoxyphosphinoyl)-5-(tertbutyldimethylsilyloxy)pentanoic acid (3.7 g; example 1) in 82 ml of anhydrous tetrahydrofuran are added, under nitrogen atmosphere and at 0°C, 1.42 g of N-hydroxysuccinimide. A solution of morpholinoethyl isonitrile (1.73 g) in 1 ml of tetrahydrofuran is added dropwise, then the temperature is raised to room temperature and the reaction mixture is kept 20 hours under stirring. The solvent is then evaporated off under reduced pressure and the residue is redissolved in ethyl

acetate (100 ml), then the organic phase is washed with 1 N hydrochloric acid (50 ml), after that with a sodium hydrogen carbonate saturated solution (50 ml), finally with a saturated solution of sodium chloride (50 ml). The organic phase is dried over sodium sulfate and the solvent is evaporated off under reduced pressure, obtaining 4 g of product.

EXAMPLE 7 - 4-[bis(2-chloroethyl)amino]-N-[5',5'-bis(dimethoxyphosphinoyl)-5'-(tertbutyldimethylsilyloxy)-1'-pentanoyl]-(L)-phenylalanine

To a suspension of 4-[bis(2-chloroethyl)amino]-(L)-phenylalanine (melphalan; 1.85 g) in 5.6 ml of water, 28 ml of acetonitrile and 1.24 g of triethylamine is slowly added dropwise (during about 1 hour) a solution of the product of example 6 (3 g) in 28 ml of acetonitrile. At the end of the dropping, the reaction mixture is kept under stirring at room temperature for 40 minutes, then the solvent is evaporated off under reduced pressure and the residue is redissolved in 100 ml of ethyl acetate. The organic phase is washed with 1 N hydrochloric acid (2x40 ml) and with water (2x40 ml), then it is dried over sodium sulfate and the solvent is evaporated off under reduced pressure. 3.7 g of product are obtained.

N.M.R. (200 MHz) in d_6-DMSO: 12 ppm (b, 1H); 8.1 ppm (d, 1H); 7.05 ppm (d, 2H); 6.65 ppm (d, 2H); 4.32 ppm (m, 1H); 3.6-3.85 ppm (m, 20H); 2.65-3 ppm (m, 2H); 1.6-2.2 ppm (m, 6H); 0.85 ppm (s, 9H); 0.15 ppm (s, 6H).

EXAMPLE 8 - 4-[bis(2-chloroethyl)amino]-N-[5',5'-diphospho-5'-(tertbutyldimethylsilyloxy)-1'-pentanoyl]-(L)phenylalanine

To a solution of the product of example 7 (2.7 g)

in 20 ml of anhydrous acetonitrile, kept under stirring and under nitrogen atmosphere at 0°C, 5 g of trimethylsilyl bromide are added. The temperature is then raised to room temperature and the stirring is kept for a total of 4 hours 30 minutes. The solvent is evaporated off under reduced pressure and the residue is partitioned between water (100 ml) and ethyl acetate (100 ml). The organic phase is separated and dried over sodium sulfate, then the solvent is evaporated off under reduced pressure, obtaining 1.88 g of the product.

N.M.R. (200 MHz) in d_6-DMSO: 8.06 ppm (d, 1H); 7.05 ppm (d, 2H); 6.65 ppm (d, 2H); 4.3 ppm (m, 1H); 3.7 ppm (s, 8H); 2.65-3 ppm (m, 2H); 1.6-2.2 ppm (m, 6H); 0.85 ppm (s, 9H); 0.15 ppm (s, 6H).

EXAMPLE 9 - 4-[bis(2-chloroethyl)amino]-N-[5',5'-diphospho-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine trisodium salt

To a solution of 2.7 g of the product of example 8 in 105 ml of acetonitrile are added 1.65 ml of 37% hydrochloric acid and the reaction mixture is kept under stirring at room temperature for 28 hours. The reaction mixture is evaporated and the residue is partitioned between 90 ml of water and 100 ml of ethyl acetate. The aqueous phase is separated and treated with a 20% sodium hydroxide solution until pH = 4.8, then the opalescent solution is cooled to 0°C and added with 250 ml of ethanol. A white solid separates which is kept at 0°C under stirring for about 50 minutes. After filtration and drying under vacuum at 50°C, 1.55 g of the product are obtained.

N.M.R. (200 MHz) in D_2O + DCl (pH = 1): 7.55 ppm (s,

4H); 4.67 ppm (dd, 1H); 4.1 ppm (t, 4H); 3.65 ppm (t, 4H); 3.35 ppm (dd, 1H); 3.05 ppm (dd, 1H); 1.7-2.35 ppm (m, 6H).

EXAMPLE 10

According to the methods described in examples 6-9, starting from the intermediates of example 2 and from the suitable substituted aminoacids, the following gem-diphosphonic acids are prepared:

4-[bis(2-chloroethyl)amino]-N-[4',4'-diphospho-4'-hydroxy-1'-butanoyl]-(L)-phenylalanine trisodium salt;

4-[bis(2-chloroethyl)amino]-N-[3',3'-diphospho-3'-hydroxy-1'-propanoyl]-(L)-phenylalanine trisodium salt;

4-[bis(2-chloroethyl)amino]-N-[8',8'-diphospho-8'-hydroxy-1'-octanoyl]-(L)-phenylalanine trisodium salt;

4-[bis(2-chloroethyl)amino]-N-[7',7'-diphospho-7'-hydroxy-1'-heptanoyl]-(L)-phenylalanine trisodium salt;

4-[bis(2-chloroethyl)amino]-N-[6',6'-diphospho-6'-hydroxy-1'-hexanoyl]-(L)-phenylalanine trisodium salt;

4-[N'-aziridinyl]-N-[5',5'-diphospho-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine trisodium salt;

4-[bis(2-chloroethyl)amino]-N-[4',4'-diphospho-4'-hydroxy-1'-butanoyl]-(L)-phenylalanine, methyl ester disodium salt;

4-[bis(2-chloroethyl)amino]-N-[3',3'-diphospho-3'-hydroxy-1'-propanoyl]-(L)-phenylalanine, ethyl ester disodium salt.

EXAMPLE 11

According to the methods described in examples 6, 7 and 9, starting from the intermediates of example 2 and from the suitable substituted aminoacids, the following esters of bis-phosphonic acids are prepared:

4-[bis(2-chloroethyl)amino]-N-[3',3'-(bis-dimethoxy-phosphinoyl)3'-hydroxy-1'-propanoyl]-(L)-phenylalanine;

4-[bis(2-chloroethyl)amino]-N-[8',8'-(bis-dimethoxy-phosphinoyl)-8'-hydroxy-1'-octanoyl]-(L)-phenylalanine;

4-[bis(2-chloroethyl)amino]-N-[7',7'-(bis-dimethoxy-phosphinoyl)-7'-hydroxy-1'-heptanoyl]-(L)-phenylalanine;

4-[bis(2-chloroethyl)amino]-N-[6',6'-(bis-dimethoxy-phosphinoyl)-6'-hydroxy-1'-hexanoyl]-(L)-phenylalanine;

4-[N'-aziridinyl]-N-[5',5'-(bis-dimethoxyphosphinoyl)-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine;

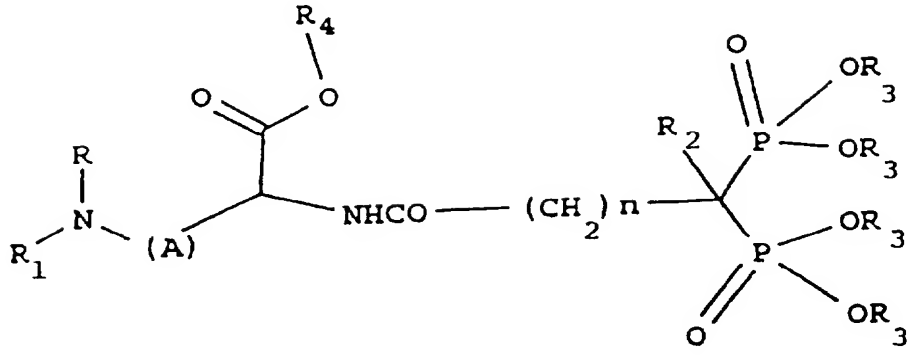
4-[bis(2-chloroethyl)amino]-N-[4',4'-(bis-dimethoxy-phosphinoyl)-4'-hydroxy-1'-butanoyl]-(L)-phenylalanine, methyl ester;

4-[bis(2-chloroethyl)amino]-N-[3',3'-(bis-dimethoxy-phosphinoyl)-3'-hydroxy-1'-propanoyl]-(L)-phenylalanine, ethyl ester;

4-[bis(2-chloroethyl)amino]-N-[5',5'-(bis-diethoxy-phosphinoyl)-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine.

CLAIMS

1. Compounds of general formula (I):

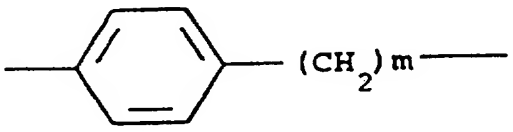


(I)

wherein:

R, R_1 halo-ethyl (2-chloroethyl, 2-bromoethyl, 2-iodoethyl) or, taken together with the nitrogen atom to which they are linked, are a 1-aziridinyl residue;

(A) is linear or branched (C_1-C_5)alkylene, phenylene or an aralkyl group of formula



wherein m is an integer from 1 to 5;

n is an integer from 1 to 6;

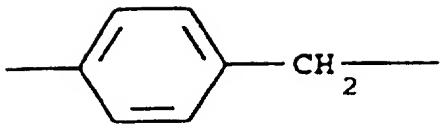
R_2 is hydrogen or a hydroxy group;

R_3 is hydrogen or (C_1-C_4)alkyl;

R_4 is hydrogen or (C_1-C_4)alkyl,

their diastereoisomers, racemic mixtures and pure enantiomers and salts thereof with pharmaceutically acceptable acids or bases.

2. Compounds according to claim 1, wherein (A) is a group of formula



3. Compounds according to claim 2, wherein R_3 and R_4 are hydrogen.

4. Compounds according to claim 3, wherein n is the integer 2 or 3 and R and R_1 are a 2-chloroethyl group.

5. Compounds according to claims 1-4, wherein R_2 is a hydroxy group.

6. Compound according to claim 1, selected in the group comprising:

4-[bis(2-chloroethyl)amino]-N-[4',4'-diphospho-1'-butanoyl](L)phenylalanine disodium salt;

4-[bis(2-chloroethyl)amino]-N-[5',5'-diphospho-1'-pentanoyl]-(L)-phenylalanine disodium salt;

4-[bis(2-chloroethyl)amino]-N-[8',8'-diphospho-1'-octanoyl]-(L)-phenylalanine disodium salt;

4-[bis(2-bromoethyl)amino]-N-[4',4'-diphospho-1'-butanoyl]-(L)-phenylalanine disodium salt;

4-[bis(2-chloroethyl)amino]-N-[3',3'-diphospho-1'-propanoyl]-(L)-phenylalanine disodium salt;

4-[N'-aziridinyl]-N-[4',4'-diphospho-1'-butanoyl]-(L)-phenylalanine disodium salt;

4-[bis(2-chloroethyl)amino]-N-[5',5'-diphospho-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine trisodium salt;

4-[bis(2-chloroethyl)amino]-N-[4',4'-diphospho-4'-hydroxy-1'-butanoyl]-(L)-phenylalanine trisodium salt;

4-[bis(2-chloroethyl)amino]-N-[3',3'-diphospho-3'-hydroxy1'-propanoyl]-(L)-phenylalanine trisodium salt;

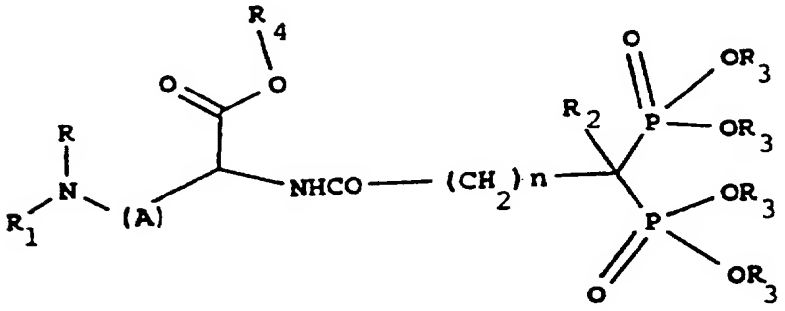
4-[bis(2-chloroethyl)amino]-N-[8',8'-diphospho-8'-hydro-

xy-1'-octanoyl]-(L)-phenylalanine trisodium salt;
4-[bis(2-chloroethyl)amino]-N-[7',7'-diphospho-7'-hydroxy-1'-heptanoyl]-(L)-phenylalanine trisodium salt;
4-[bis(2-chloroethyl)amino]-N-[6',6'-diphospho-6'-hydroxy-1'-hexanoyl]-(L)-phenylalanine trisodium salt;
4-[N'-aziridinyl]-N-[5',5'-diphospho-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine trisodium salt;
4-[bis(2-chloroethyl)amino]-N-[4',4'-diphospho-4'-hydroxy-1'-butanoyl]-(L)-phenylalanine, methyl ester disodium salt;
4-[bis(2-chloroethyl)amino]-N-[3',3'-diphospho-3'-hydroxy-1'-propanoyl]-(L)-phenylalanine, ethyl ester disodium salt;
4-[bis(2-chloroethyl)amino]-N-[3',3'-(bis-dimethoxyphosphinoyl)-3'-hydroxy-1'-propanoyl]-(L)-phenylalanine;
4-[bis(2-chloroethyl)amino]-N-[8',8'-(bis-dimethoxyphosphinoyl)-8'-hydroxy-1'-octanoyl]-(L)-phenylalanine;
4-[bis(2-chloroethyl)amino]-N-[7',7'-(bis-dimethoxyphosphinoyl)-7'-hydroxy-1'-heptanoyl]-(L)-phenylalanine;
4-[bis(2-chloroethyl)amino]-N-[6',6'-(bis-dimethoxyphosphinoyl)-6'-hydroxy-1'-hexanoyl]-(L)-phenylalanine;
4-[N'-aziridinyl]-N-[5',5'-(bis-dimethoxyphosphinoyl)-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine;
4-[bis(2-chloroethyl)amino]-N-[4',4'-(bis-dimethoxyphosphinoyl)-4'-hydroxy-1'-butanoyl]-(L)-phenylalanine, methyl ester;
4-[bis(2-chloroethyl)amino]-N-[3',3'-(bis-dimethoxyphosphinoyl)-3'-hydroxy-1'-propanoyl]-(L)-phenylalanine, ethyl ester;
4-[bis(2-chloroethyl)amino]-N-[5',5'-(bis-diethoxyphosphinoyl)-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine.

7. Compound according to claim 6, wherein such compound is:

4-[bis(2-chloroethyl)amino]-N-[5',5'-diphospho-5'-hydroxy-1'-pentanoyl]-(L)-phenylalanine trisodium salt.

8. A process for the preparation of the compounds of formula (I):

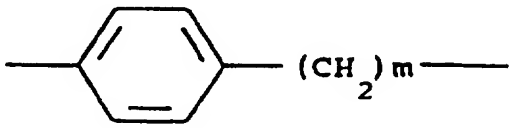


(I)

wherein:

R, R_1 halo-ethyl (2-chloroethyl, 2-bromoethyl, 2-iodoethyl) or, taken together with the nitrogen atom to which they are linked, are a 1-aziridinyl residue;

(A) is linear or branched (C_1-C_5)alkylene, phenylene or an aralkyl group of formula



wherein m is an integer from 1 to 5;

n is an integer from 1 to 6;

R_2 is hydrogen or a hydroxy group;

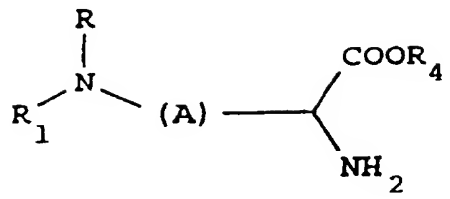
R_3 is hydrogen or (C_1-C_4)alkyl;

R_4 is hydrogen or (C_1-C_4)alkyl,

their diastereoisomers, racemic mixtures and pure enantiomers and salts thereof with pharmaceutically acceptable acids or bases, comprising the following

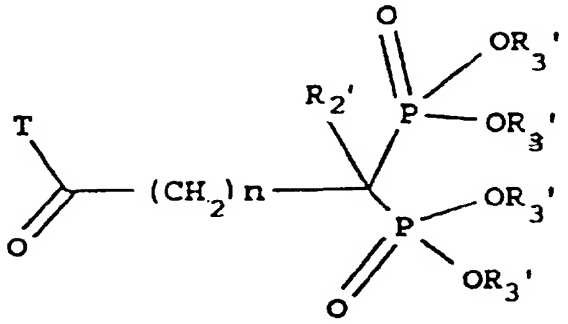
steps:

(a) condensation reaction of the compounds of formula (II):



(II)

wherein R, R_1, (A) and R_4 have the above meanings, with a bis-phosphonate of formula (III):



(III)

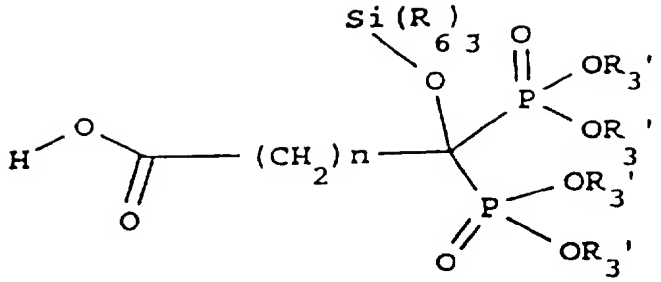
wherein n has the above meaning, R_2' has the above meanings or is a group O-G, in which G is a suitable protecting group for a tertiary alcohol, R_3' has the above meanings except hydrogen, T is a hydroxy group or a group which activates the carboxylic functionality.

(b) selective removal of the protecting groups optionally present and/or optional hydrolysis of the phosphonic esters to give the corresponding acids;

(c) optional salification with pharmaceutically acceptable acids or bases;

(d) optional separation of diastereoisomers or enantiomers.

9. Compounds of formula (VIII):



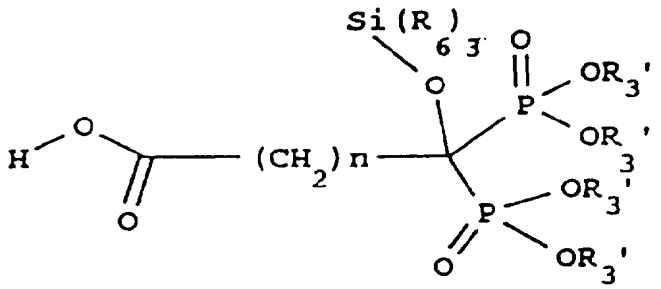
(VIII)

wherein n is an integer from 1 to 6, R_3' is linear or branched (C_1-C_4)alkyl and the R_6 groups, which can be the same or different, are selected from the group comprising linear or branched (C_1-C_4)alkyl and phenyl group, as intermediates.

10. Compound according to claim 9, wherein said compound is:

5,5-bis(dimethoxyphosphinoyl)-5-(tertbutyldimethylsilyl-oxy)pentanoic acid.

11. A process for the preparation of the compounds of formula (VIII):



(VIII)

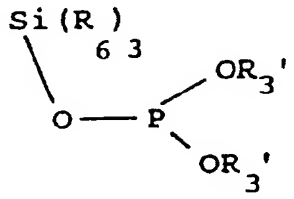
wherein n is an integer from 1 to 6, R_3' is linear or branched (C_1-C_4)alkyl and the R_6 groups, which can be the same or different, are selected in the group comprising linear or branched (C_1-C_4)alkyl and phenyl group, comprising the following steps:

(a) reaction of a cyclic anhydride of a suitable dicarboxylic acid with an alcohol of formula R_5-OH, wherein R_5 is (C_1-C_4)alkyl, optionally substituted benzyl or allyl;

(b) activation of the free carboxylic group of the compound obtained in step (a);

(c) reaction of the intermediate obtained on step (b) with a trialkyl phosphite $P(OR_3')_3$, wherein R_3' has the above meanings, to give a keto-phosphonic ester;

(d) reaction of the compound obtained in step (c) with a dialkyl silyl phosphite of formula (VII):



(VII)

wherein R_3' and R_6 have the above meanings, followed by the hydrolysis of the carboxylic ester group, to give the compounds of formula (VIII).

12. A process according to claim 11, wherein the R_5 group is a benzyl group and wherein the hydrolysis of the step of the carboxylic group in step (d) is performed by means of catalytic hydrogenation.

13. Process according to claim 12, wherein the -Si(R_6)$_3$ group is a tertbutyldimetylsilyl group.

14. Pharmaceutical compositions containing at least one compound according to claims 1-7 as active ingredient, together with pharmaceutically acceptable excipients.

15. The use of the compounds according to claims 1-7 as antitumor and anti-osteolytic agents.

16. The use according to claim 15, in which the tumors to be treated are multiple myeloma, osteosarcoma, bone metastasis, breast, ovary and testis carcinomas.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 97/03190

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07F9/38 A61K31/66 C07F9/40 C07F9/564

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07F A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 170 896 A (HENKEL KGAA ;DEUTSCHES KREBSFORSCH (DE)) 12 February 1986 cited in the application see the whole document ---	1-8, 14-16
Y	WO 88 06158 A (STURTZ GEORGES) 25 August 1988 cited in the application see the whole document ---	1-8, 14-16
Y	WO 92 18512 A (BOEHRINGER MANNHEIM ITALIA) 29 October 1992 cited in the application see the whole document ---	1-8, 14-16
Y	WO 91 05791 A (BOEHRINGER BIOCHEMIA SRL) 2 May 1991 see the whole document ---	1-8, 14-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 October 1997	17. 10. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Beslier, L

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INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/EP 97/03190

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 090, no. 15, 9 April 1979 Columbus, Ohio, US; abstract no. 115081, BANDURINA T A ET AL: "Synthesis of some aminophosphonic acids and their antineoplastic activity" XP002023938 see abstract & KHIM.-FARM. ZH. (KHFZAN,00231134);78; VOL.12 (11); PP.35-7, URAL. POLITEKH. INST.;SVERDLOVSK; USSR,	1-8, 14-16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 97/03190

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0170896 A	12-02-86	DE 3425812 A	16-01-86
		JP 1961188 C	10-08-95
		JP 6051710 B	06-07-94
		JP 61063687 A	01-04-86
		US 4608368 A	26-08-86
WO 8806158 A	25-08-88	FR 2611203 A	26-08-88
WO 9218512 A	29-10-92	IT 1246777 B	26-11-94
		AT 144986 T	15-11-96
		AU 1459192 A	17-11-92
		DE 69215085 D	12-12-96
		DE 69215085 T	03-04-97
		EP 0580642 A	02-02-94
		ES 2096076 T	01-03-97
		JP 6506452 T	21-07-94
		US 5446187 A	29-08-95
WO 9105791 A	02-05-91	IT 1241674 B	27-01-94
		AT 137757 T	15-05-96
		AU 639495 B	29-07-93
		AU 6510890 A	16-05-91
		CA 2067191 A	13-04-91
		DE 69026964 D	13-06-96
		DE 69026964 T	24-10-96
		EP 0593437 A	27-04-94
		ES 2090143 T	16-10-96
		JP 5500949 T	25-02-93
		US 5300671 A	05-04-94